



Application No.: 08/846,658
Title: Humanised Antibodies
Inventors: John R. Adair, Diljeet S. Athwal and John S. Emtage
Docket No. CARP-0057
Date of Filing: May 1, 1997
Atty: Doreen Yatko Trujillo
Telephone No. (215) 568-3100

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TECH CENTER 1600/2900

GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg 1 ctaatcagta cctcagtcat aatatccaga agacaaatta ttctcaccca 51 atctccagca atcatatcta catctccago agagaaggtc accatgacct 101 gragtgrag ctraagtgta agttaratga artggtarra gragaagtra 151 ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg 201 agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctca 251 caatcagegg catggagget gaagatgetg ceaettatta etgecageag 301 tggagtagta accattcac gttcggctcg gggacaaagt tggaaataaa 351 ccgggctgat actgcaccaa-ctgtatccat cttcccacca tccagtgagc 401 agttaacate togaggtgee teagtegtgt gettettgaa caacttetae 451 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa 501 tggcgtčctg aacagttgga čtgatcagga čagčaaagač agcacctaca 551 gcatgagcag cacceteaeg ttgaccaagg acgagtatga acgacataac 601 agetatacet atgaggecae teacaagaca teaactteae ecattateaa 651 gagetteaac aggaatgagt gtTAGAĞACA AAGGTCCTGA GACGCČACCA 701 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC 751 CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT 801 TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA 851 901 (SEQ ID NO:4)

FIG. 1a

1	MDFQVQIFSF	LLISASVIIS	RGQQIVLTQSF	A I MSASPGEK	VTMTCSASSS
51	VSYMNWYQQK	SGTSPKRWIY	DTSKLASGVP	AHFRGSGSGT	SYSLTISGME
	AEDAATYYCQ				PSSEQLTSGG
				LNSWTDQDSK	
201	TI TKDEYERH	NSYTCEATHK	TSTSPIVKSF	NRNEC* (SEQ	ID ND:5)

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GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC 1 ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG 51 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT 101 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC 151 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT 201 ATTCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC 251 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA 301 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT 351 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC 401 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG 451 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT 501 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG 551 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA 601 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC 651 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC 701 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT 801 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT 851 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG 901 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT 951 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG 1001 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG 1051 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT 1101 GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC 1151 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA 1201 ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC 1251 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA 1301 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC 1351 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT 1401 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA 1451 TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA 1501 (SEQ ID NO:6) AAAAAAAAA AAAGGAATTC 1551

FIG. 2a

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OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

1	MERHWIFLLL	LSVTAGVHSQ	VQLQQSGAEL	ARPGASVKMS	CKASGYIFIR
51	YTMHWVKQRP	GQGLEWIGYI	NPSRGYTNYN	QKFKDKATLT	TDKSSSTAYM
101	QLSSLTSEDS	AVYYCARYYD	DHYCLDYWGQ	GTTLTVSSAK	TTAPSVYPLA
151	PVCGDTTGSS	VTLGCLVKGY	FPEPVTLTWN	SGSLSSGVHT	FPAVLQSDLY
201	TLSSSVTVTS	STWPSQSITC	NVAHPASSTK	VDKK I EPRGP	TIKPCPPCKC
251	PAPNLLGGPS	VFIFPPKIKD	VLMISLSPIV	TCVVVDVSED	DPDVQISWFV
301	NNVEVHTAQT	QTHREDYNST	LRVVSALPIQ	HQDWMSGKEF	KCKVNNKDLP
351	APIERTISKP	KGSVRAPQVY	VLPPPEEEMT	KKQVTLTCMV	TDFMPEDIYV
401	EWTNNGKTEL	NYKNTEPVLD	SDGSYFMYSK	LRVEKKNWVE	RNSYSCSVVH
451	EGLHNHHTTK				

FIG. 2b

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42 53 Ν N NN Ν SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe RES TYPE QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT □kt3vl DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQ<u>T</u>PGK **REI** CDR1 (LOOP) (KABAT) CDR1 *****

56 85

N NN

RES TYPE *Is:PpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Dkt3vl SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT

SPKRWIYDTSKLASGVPA<u>H</u>F<u>R</u>GSGSGTSYSLTIS<u>G</u>MEAEDAAT APKLLIYEASNLQAGVPSRFSGSGSGTD<u>Y</u>T<u>F</u>TISSLQPED<u>I</u>AT **(SEQ**

REI APKLLIYEASNLQAGVPSRFSGSGSGTDYIFIISSLQPEDIAI (SEID NO:8)

****** CDR2 (LOOP/KABAT)

102 108
RES TYPE PiPIPies**iPIIsPPSPSSS

Okt3vl YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)

REIVI YYCQQYQSLPYTFGQGTK<u>LQ</u>I<u>T</u>R (SEQ ID NO:9)

****** CDR3 (LOOP)

********* CRD3(KABAT)

FIG. 3

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32 35 N39 23 26 NN N SESPs^SBssS^sSsSpSpSPsPSEbSBssBePi^PIpiesss RES TYPE QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMNHWVKQRPGQ Okt3h QVQLVESGGG<u>V</u>VQPG<u>R</u>SLRLSC<u>SS</u>SGF<u>I</u>FSSYAMYWVRQAPGK K0L CDR1 (LOOP) ***** CDR1 (KABAT) **** N N N82abc 52a 60 65 RES TYPE IIeIppp^ssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb GLEWIGYINPSRGYTNTNQKFKRKATLTTDKSSSTAYMQLSSLTSEDSAV GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLPPEDTGV KOL CDR2 (LOOP) ***** CDR2 (KABAT) ****** 107 113 92 N PiPIEissssiiisssbibi*EIPIP*spSBSS RES TYPE (SEQ ID ND:30) YYCARYYDDHY.....CLDYWGQGTTLTVSS □kt3vh YFCARDGGHGFCSSASCFGPDYWGQGT<u>P</u>VTVSS (SEQ ID NO:10) KOL ****** CRD4 (KABAT/LOOP)

FIG. 4

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OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

	1	26	35	39	43	
□kt3vh	QVQLQQSGAELARPGASVKM	SCKASGYTFTRY	TMHW	VKQRI	≥GQ	•
gH341	QVQLVESGGGVVQDGRSLRL					JA178
gH341A	QVQLV <u>Q</u> SGGGVVQPGRSLRL	SC <u>KASGYTFTRY</u>	<u>TM</u> HW	VRQA	PGK	JA185
	OVER VERSEEN VERSERS BL	CCUACCUTETON	T 641 11 2	VDO 41	רע.	14100
gH341E	QVQLVQSGGGVVQPGRSLRL					JA198
gH341*	QVQLVQSGGVVQPGRSLRL					JA207
gH341*	QVQLVQSGGVVQPGRSLRL					JA209
gH341D	QVQLVQSGGVVQPGRSLRL	SCKASGYTFTRY	<u>TM</u> HW	VRQA	PGK	JA197
gH341*	QVQLVQSGGGVVQPGRSLRL	SCKASGYTFTRY	TMHW	VRQA	PGK	JA199
gH341C	QVQLVQSGGGVVQPGRSLRL	SC <u>KASGYTFTR</u> Y	<u>TM</u> HW	VRQA	PGK	JA184
J	_					
gH341*	QVQLVQSGGGVVQPGRSLRL	SCS <u>ASGYTFTR</u>)	<u> 'TM</u> HW	VRQA	PGK	JA203
gH341*	QVQLVESGGGVVQPGRSLRL	SCSASGYTFTRY	TMHW	VRQA	PGK	JA205
gH341B	QVQLVESGGGVVQPGRSLRL					JA183
gH341*	QVQLVQSGGGVVQPGRSLRL					JA204
gH341*	QVQLVESGGGVVQPGRSLRL					JA206
gH341*	QVQLVQSGGGVVQPGRSLRL	SCSASGYTETRY	/TMHW	VRDA	PGK	JA208
•	QVQLVESGGGVVQPGRSLRL	202 <u>727 10</u>	<u>····</u> ···ν ΔΜΥW	VPOA	PGK	3200
KOL	WAME AEROGRA AMERIKRE	3033301 11 33	mull W	4 I/ OX I	· UIX	

FIG. 5a

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	44	50	65	. 83	
□kt3vh	GLEW	IGYINPSRG	YTNYNQKFKDKATLTTDK	SSSTAYMQLSSLT	•
gH341	GLEW	VAYINPSRG	<u>YTNYNQKFKD</u> RFTISRDN	SKNTLFLQMDSLR	JA178
gH341A	GLEW	<u>IGY INPSRG</u>	YTNYNQKVKDRFTIS <u>T</u> D <u>K</u>	SKSTAFLQMDSLR	JA185
gH341E	GLEW	IGYINPSRG	<u>YTNYNQK</u> VK <u>D</u> RFTIS <u>I</u> D <u>K</u>	SKSTAFLQMDSLR	JA198
gH341*	GLEW	IGYINPSRG	<u>YTNYNQK</u> VK <u>D</u> RFTIS <u>T</u> DK	SKNTÆFLQMDSLR	JA207
gH341*			<u>YTNYNOK</u> VK <u>D</u> RFTISRDÑ		JA209
gH341D	GLEW	IGYINPSRG	<u>YTNYNQK</u> VK <u>D</u> RFTIS <u>T</u> DK	SKNTĒFLQMDSLR	JA197
gH341*	GLEW	IGYINPSRG	<u>YTNYNQK</u> VK <u>D</u> RFTISRDN	SKNTLFLQMDSLR	JA199
gH341C	GLEW	VAY I NPSRG	<u>YTNYNQKFKD</u> RFTISRDN	SKNTLFLQMDSLR	JA184
gH341*	GLEW	IGYINPSRG	<u>YTNYNOK</u> VK <u>D</u> RFTIS <u>T</u> D <u>K</u>	SK <u>S</u> TAFLQMDSLR	JA207
gH341*	GLEW	ĪĠYINPSRG	YTNYNOKVKDRFTISĪDK	SKSTAFLQMDSLR	JA205
gH341B	GLEW	ĪĠYINPSRG	YTNYNOKVKDRFTIS <u>T</u> DK	SKĪTĀFLQMDSLR	JA183
gH341*	GLEW	IGYINPSRG	YTNYNDKVKDRFTISTD <u>K</u>	SKĪTĀFLQMDSLR	JA204
gH341*	GLEW	IGYINPSRG	YTNYNOKVKDRFTISTDK	SKĪTĀFLQMDSLR	JA206
gH341*	GLEW	IGYINPSRG	<u>YTNYNOK</u> V <u>KD</u> RFTISĪD <u>Ē</u>	SKÑTÆFLQMDSLR	80 SAL
КOL	GLEW	VA I I WDDGS	DQHYADSVKGRFTISRDN	ISKNTĒFLQMDSLR	

FIG. 5b

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SEQ ID ND: 1 02 84 95 SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS Okt3vh PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS 30 JA178 gH341 PEDTAVYYCARYYDDHY.....DLDYWGQGTTLTVSS JA185 12 qH341A PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS JA198 13 9H341E JA207 14 PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS gH341* JA197 15 PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS **QH341D** PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS JA209 16 qH341* JA199 PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS 17 qH341* JA184 18 PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS gH341C 19 PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS JA203 qH341* JA205 20 qH341* 21 PEDTĀVY\CARYYDDHY.....CLDYWGQGTTLTVSS JA183 9H341B PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS JA204 55 gH341* JA206 23 PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS gH341*

805AL

24

10

FIG. 5c

PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS

PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS

qH341*

KOL

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DKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

Okt3vl gL221 gL221A gL221B gL221C REI	1 QIVLTQSPADMSASPGEKVTI DIQMTQSPSSLSASVGDRVT QIVMTQSPSSLSASVGDRVT QIVMTQSPSSLSASVGDRVT DIQMTQSPSSLSASVGDRVT DIQMTQSPSSLSASVGDRVT	I TC <u>SASS . S</u> I TC <u>SASS . S</u> I TC <u>SASS . S</u> I TC <u>SASS . S</u>	VSYMNWYQQ VSYMNWYQQ VSYMNWYQQ VSYMNWYQQ	TPGK TPGK TPGK TPGK			
Okt3vl gL221 gL221A gL221B gL221C REI	43 50 56 SPKRWIYDTSKLASGVPAHF APKLLIY <u>DTSKLAS</u> GVPSRF APK <u>RWIYDTSKLAS</u> GVPSRF APK <u>RWIYDTSKLAS</u> GVPSRF APK <u>RWIYDTSKLAS</u> GVPSRF APK <u>RWIYDTSKLAS</u> GVPSRF APKLLIYEASNLQAGVPSRF	YGTB2B2B2 YGTB2B2B2 YGTB2B2B2 YGTB2B2B2	TFTISSLQP TFTISSLQP TFTISSLQP TFTISSLQP	EDIAT EDIAT EDIAT EDIAT		ID NO:8	>
Okt3vl gL221 gL221A gL221B gL221C REI		ITR ITR ITR ITR	(SEQ ID N (SEQ ID N (SEQ ID N	ND: 29) ND: 25) ND: 26) ND: 27) ND: 28) ND: 9)	Z."		

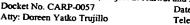
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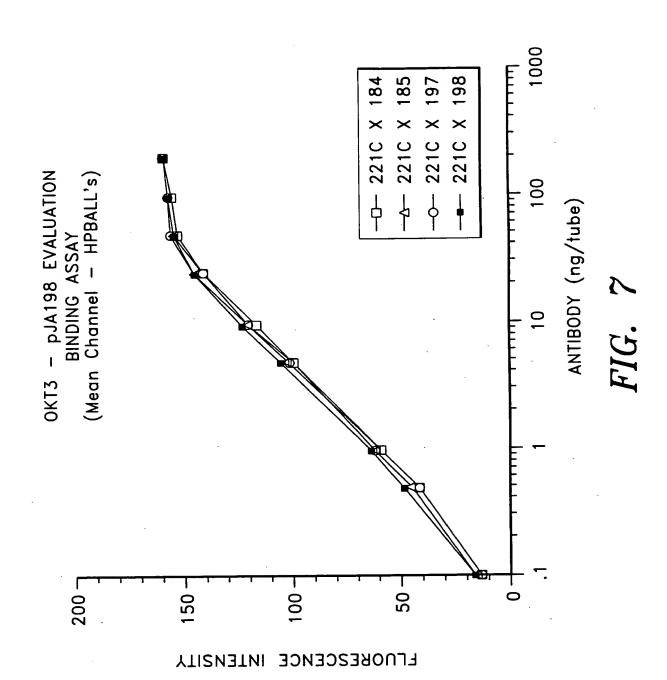
FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

FIG. 6

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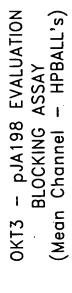


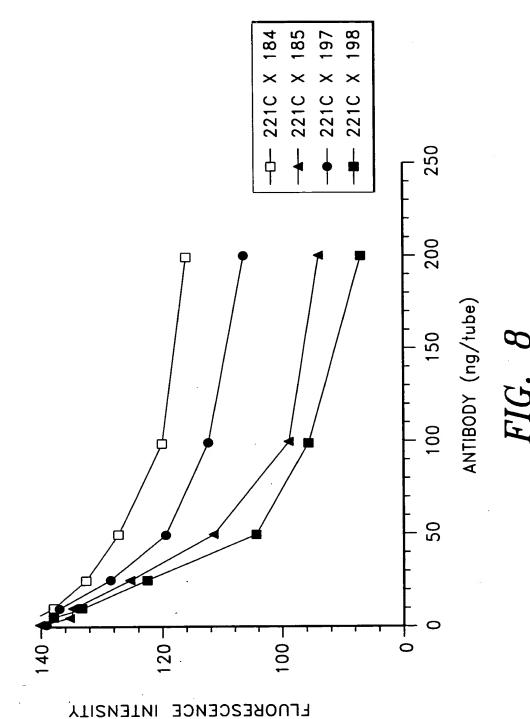


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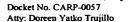
Atty: Doreen Yatko Trujillo

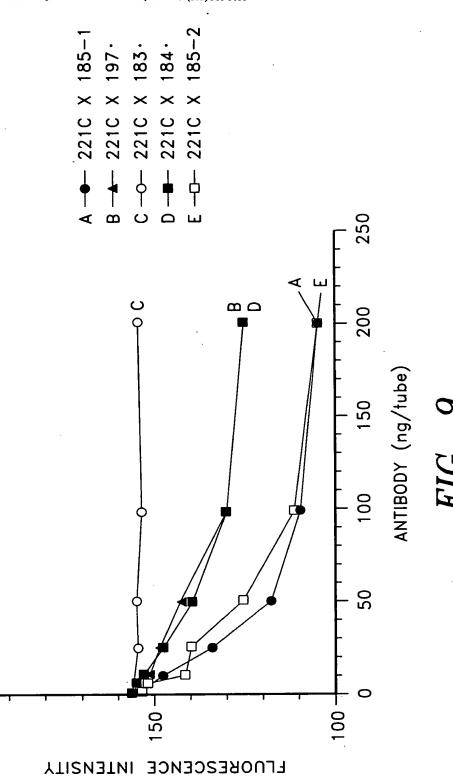
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BLOCKING ASSAY (Mean Channel — HPBALL's)

200기

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OKT3 - GRAFTED HEAVY CHAINS BINDING ASSAY (Mean Channel - HPALL's) 200 FLUORESCENCE INTENSITY 141 221C 185 В X 221C 150 X 199 C 221C X 204 D 221C X 205 Ε 100 221C X 207 F 221C 208 G X 50 H 221C X 209 0 100 1000 10 ANTIBODY (ng/tube)

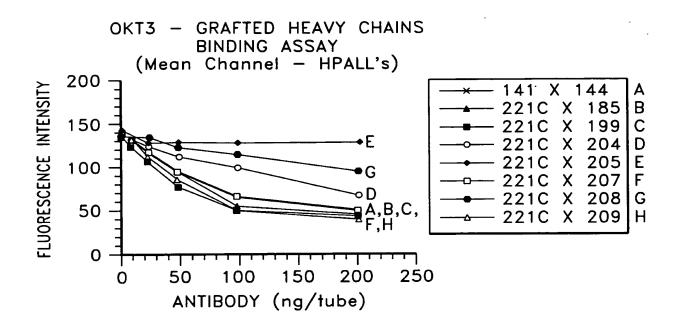
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-- (205) _,__,24,48,49,71,73,76,78,88,91,
-- (208) 6,__,24,48,49,71,73,__,78,__,,
-- (204) 6,__,24,48,49,71,73,76,78,__,,
-- (199) 6,23,24,48,49,__,,__,71,73,,76,78,,__,,
-- (207) 6,23,24,48,49,71,73,76,78,88,91,
-- (185) 6,23,24,48,49,71,73,76,78,88,91,
-- (209) 6,23,24,48,49,__,_,_,78,__,,
-- (141 X 144
```

FIG. 10a

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--- (205) _,__,24,48,49,71,73,76,78,88,91,
--- (208) 6,__,24,48,49,71,73,__,78,__,,
--- (204) 6,__,24,48,49,71,73,76,78,__,,
--- (199) 6,23,24,48,49,
--- (207) 6,23,24,48,49,71,73,76,78,88,91,
--- (185) 6,23,24,48,49,71,73,76,78,88,91,
--- (209) 6,23,24,48,49,__,_,_,78,__,
--- (141 X 144
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FIG. 10b

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OKT3 - GRAFTED HEAVY CHAINS BINDING ASSAY (Mean Channel - HPALL's)

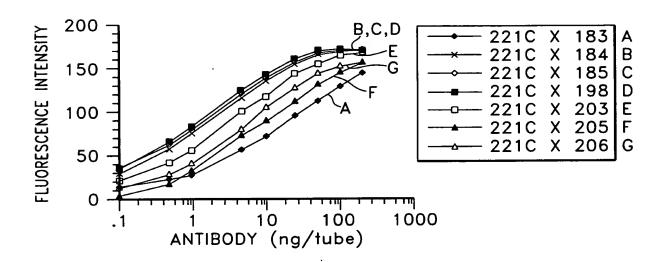


FIG. 11a

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OKT3 - GRAFTED HEAVY CHAINS BINDING ASSAY (Mean Channel - HPALL's)

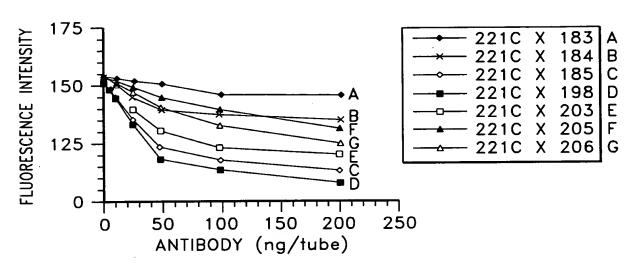
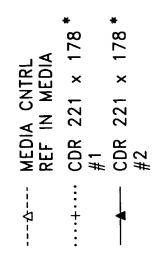
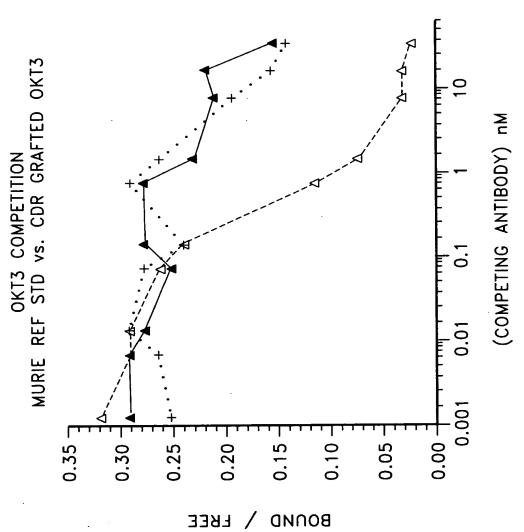


FIG. 11b





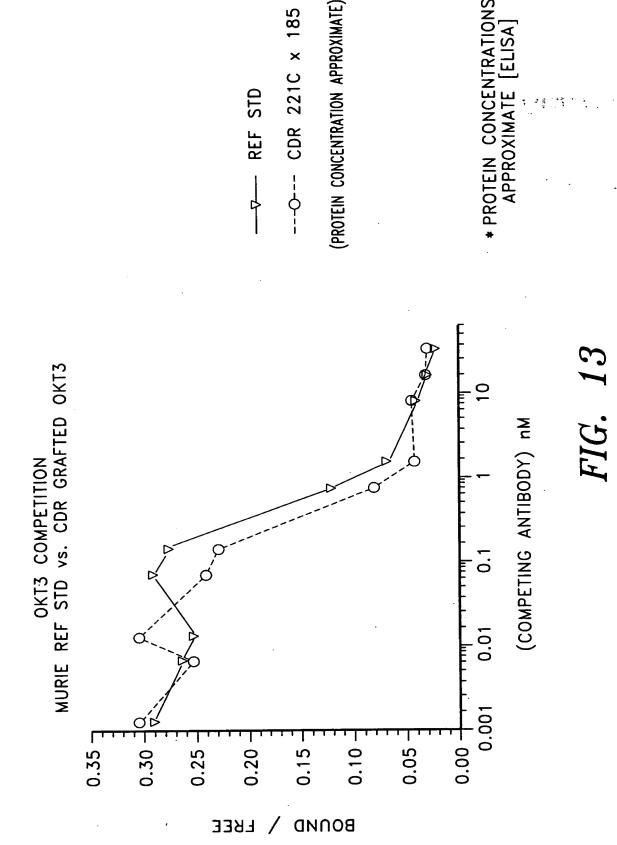


REF STD

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* PROTEIN CONCENTRATIONS APPROXIMATE [ELISA]

4